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Original Article

Stress Questionnaires and Stress Biomarkers during Pregnancy

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Abstract

Objective: Both self-reported indicators of stress and hormones such as cortisol and corticotrophin-releasing hormone (CRH) have been examined in relation to preterm birth. Although these hormones have been interpreted as biomarkers of stress, it is unclear whether psychosocial measures are empirically associated with biomarkers of stress in pregnant women.

Methods: We analyzed data from 1,587 North Carolina pregnant women enrolled in the Pregnancy, Infection, and Nutrition study during 2000–2004 who provided at least one saliva sample for cortisol measurement or blood samples for CRH at 14–19 and 24–29 weeks' gestation. Cortisol measures were limited to those taken between 8 and 10 a.m. Perceived stress, state-trait anxiety, coping style, life events, social support, and pregnancy-specific anxiety were measured by questionnaires and interviews. Spearman correlations and multiple regressions were used to describe the relationship among the measures of stress.

Results: No correlations larger than $r = 0.15$ were seen between reported psychosocial measures and cortisol or CRH. Women with demographic characteristics associated with poor pregnancy outcomes (unmarried, African-American, young, low pre-pregnancy body mass index) reported higher levels of stress but did not consistently have higher levels of stress hormones. Pre-eclampsia was associated with higher CRH, but not with higher cortisol.

Conclusions: The relationship between measurements of reported stress and biomarkers is not straightforward in large epidemiological studies of pregnancy. For online Supplementary Material, see www.liebertonline.com.

Introduction

Many studies have suggested that stress and stress hormones have a role in the etiology of preterm birth (PTB).[1] It has been hypothesized that stress increases levels of cortisol and corticotrophin-releasing hormone (CRH), and increased CRH causes PTB.[2,3] Under stress-inducing laboratory challenges, the hypothalamic-pituitary-adrenal axis responds by releasing both cortisol and CRH.[4] During pregnancy, cortisol stimulates the production of CRH in the placenta,[5] and both cortisol[6] and CRH have been found to be higher in medically complicated pregnancies in several,[7,8] though not all,[9] studies.

However, few studies have examined the links in this chain directly. Hobel et al. reported an association between stress and CRH, between CRH and plasma cortisol, and between CRH and PTB, but the associations between perceived stress and CRH varied by whether the woman gave birth prematurely.[7] Mancuso et al. found that pregnancy-specific anxiety at 28–30 weeks was positively correlated with CRH, which was positively associated with PTB.[10] However, no association was seen between perceived stress or state anxiety, and CRH at either 18–20 or 28–30 weeks, nor was there any association between pregnancy-specific anxiety at 18–20 weeks and CRH at either point.[10] Other studies have found an association between some measures of reported stress and stress hormones;[10–12,13] however, this was not true for other psychosocial measures of stress.[10,12,14–18] Similarly, if cortisol leads to increased CRH, we would expect the two to be correlated. Although Sandman et al. reported that increased

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plasma cortisol at 15 weeks led to increased CRH at 31 weeks,[6] and Goland et al. reported a strong correlation between CRH and 24-hour free urinary cortisol,[19] another study did not show the same results.[20]

In this analysis, we explore associations between biomarkers of stress and several repeated psychosocial measures of stress and mental health in a large cohort of pregnant women (a study design commonly used to examine questions of psychosocial variables and pregnancy health). Our goals are to (1) examine the associations between cortisol and CRH measures; (2) examine the associations between psychosocial measures of stress and these biological measures of stress; and (3) determine if demographic and behavioral factors that are also markers of risk for PTB are associated with these stress measurements.

Methods

The Pregnancy, Infection, and Nutrition Study (PIN) addressed prenatal influences on pregnancy outcomes. Women who sought prenatal care before gestational week 20 at the University of North Carolina Hospitals during 2001–2004 were recruited. Exclusion criteria were not having access to a telephone (<1% ineligible), not speaking English (11% ineligible), being less than 16 years old (<1% ineligible), multiple gestations (4% ineligible), or the woman's healthcare provider did not feel it was in her best interest to participate (<2% ineligible). Before 20 weeks' gestation, women provided saliva and blood at a recruitment visit or, if they agreed to a fasting blood draw, at a research visit to the General Clinical Research Center (visit 1, 14–18 weeks). A second research visit occurred during weeks 24–29 (visit 2). Gestational age was determined via ultrasound when performed before week 22 and otherwise based on reported last menstrual period.

Lazarus' stress-coping model posits that stress is an excess of environmental demand beyond a person's ability to meet it (stressor). Stress is most commonly conceived as a person-environment transaction, the first part of which involves a threat, the second part involves the appraisal of the threat, and the third is a person's response to the threat.[21] A perceived threat is an excess of environmental demand beyond the individual's capacity to meet it, with important perceived consequences.[22] The stressors a person experiences interact with his or her psychological state, personal disposition, and social support, on the background of their physiologic substrate and the social and environmental context.[23] Most psychological measurements involve one of the subparts of this transaction: stressor, appraisal, or response. The scales were chosen to provide information on external stressors (the Sarason's Life Experiences Survey), perceived stress, enhancers of response to external stressors (Trait Anxiety Inventory, and pregnancy-specific anxiety), and buffers of response to external stressors (social support and John Henryism coping).

Psychosocial stress was measured in two telephone interviews and by two self-administered questionnaires (Table 1). Details are available online (www.cpc.unc.edu/projects/pin/docs_3/index.html). Most of the measures were widely used, validated instruments. Except where noted, psychometric data are from validation studies that were conducted on non-pregnant adult populations. A subset of 39 items from Sarason's Life Experiences Survey provided a composite score

Table 1. Measurements Taken in the Pregnancy, Infection, and Nutrition Study (PIN) Protocol

Data collection time point	*Median gestational age in weeks at collection (interquartile range)*	*Data or specimens collected pertinent to this analysis*	*Number available for this analysis (% of overall sample)*
Initial recruitment	15 (13–17)		1587
Phone interview #1[a]	19 (18–20)	Life Experiences Survey	1536 (97%)
		Perceived Stress Scale	1534 (97%)
Self-administered questionnaire #1	16 (14–18)	State-Trait Anxiety Inventory	1419 (89%)
		Medical Outcome Study Social Support Scale	1419 (89%)
		Orr pregnancy-related anxiety	1425 (90%)
Clinic visit #1[b]	17 (16–18)	Saliva for cortisol (analysis limited to those collected between 8 and 10 a.m.)	460 (29%)
	17 (16–19)	Blood for CRH	1486 (94%)
Phone interview #2	28 (27–29)	Perceived Stress Scale	1422 (90%)
		Rini pregnancy-related anxiety	1423 (90%)
Self-administered questionnaire #2	27 (26–29)	Life Experiences Survey	1241 (78%)
		State Anxiety Inventory	1233 (77%)
		John Henryism Active Coping Scale	1234 (78%)
		Orr pregnancy-related anxiety	1241 (78%)
Clinic visit #2	26 (25–28)	Saliva for cortisol (collected between 8 and 10 a.m.)	353 (22%)
		Blood for CRH	1466 (92%)

[a]Completion of this interview was required to continue in the study.
[b]This clinic visit was optional for the basic PIN protocol. Women who did not participate in this part of the study provided a saliva sample at recruitment.
CRH, corticotropin-releasing hormone.

of life events and the perceived impact of those events.[24] The first interview asked about events since getting pregnant, the second about events since the first interview. For this analysis, two scales were used: the sum of the absolute value of perceived negative impacts and the sum of the total absolute values of the negative and positive perceived impacts. The scale has moderate test-retest reliability for these measures (reliability coefficients of 0.56–0.88) and correlates with depression, personal maladjustment, and academic achievement.[24] The Spielberger State-Trait Anxiety Inventory (STAI)[25] contains two 20-item scales to assess anxiety. The state anxiety scale measures current feelings of anxiety or how the respondent feels "right now," while trait anxiety is measured by questions that ask how the respondent "generally feels." Two measures of pregnancy-specific anxiety were used. Rini et al.'s scale focuses on worry about the woman's and her baby's health, labor and delivery, and caring for the baby;[26] six items specific to pregnancy health were taken from the Prenatal Social Environment Inventory of Orr et al.,[27] and four items were added. The "John Henryism" Active Coping Scale includes 12 items that measure coping, overcoming obstacles, and making one's own way in the world.[28] The Medical Outcomes Study (MOS) Social Support Survey assesses perceived social support, including questions about the availability of emotional, informational, tangible, and affectionate support.[29] Item-scale correlations are greater than 0.7, and internal consistency is high for all categories of measures, exceeding 0.50. The scale correlates with measures of loneliness, emotional ties, and family functioning.[29] Women were asked to assess this support since they became pregnant. The Cohen Perceived Stress Scale[30] is designed to measure "the degree to which situations in one's life are appraised as stressful." The 14-item scale was used at the first interview and the 10-item at the second. Reliability is between 0.84 for short-term and 0.55 for longer-term test-retest.[30]

At each clinic visit, a saliva sample was taken to measure cortisol and a blood sample to measure CRH. Samples were taken between 7:30 a.m. and 7:00 p.m. For the purposes of this analysis, we limited the cortisol sample to those taken between 8 a.m. and 10 a.m. For the saliva sample, each study participant was asked to rinse her mouth thoroughly with water 15 minutes before collection. The saliva was collected in a plastic tube and stored at -20°C as soon as possible. Blood was collected in a chilled syringe, transferred to a tube containing EDTA (1 mg/ml of blood) and Aprotinin (500 KIU/ml of blood), and centrifuged at 0°C. The plasma was decanted from the tube, aliquoted into four cryogenic storage tubes, and stored at -70°C until extraction.

Saliva samples were assayed for salivary cortisol using a high-sensitive enzyme immunoassay (Salimetrics, PA). The test uses 25 μl of saliva and has a range of sensitivity of 0.007–1.8 μg/dL; average intra- and inter-assay coefficients of variation were 4.13% and 8.89%, respectively. Eleven percent of the samples were analyzed in duplicate, and the mean of the two values was used.

Fifty μl-plasma samples were assayed for CRH using a competitive enzyme immunoassay. The assay had a minimum detection limit of 0.08 ng/mL and a range of 0–25 ng/mL. Average intra- and inter-assay coefficients of variation were <5% and <14%, respectively. Samples were assayed by Salimetrics, LLC (State College, PA). Seven percent of the samples were analyzed in duplicate, and the mean of the two values was used. Cortisol and CRH results, which were right-skewed, were log-transformed.

All protocols were approved by the UNC School of Medicine Institutional Review Board.

Statistical analysis

As shown in Table 1, the amount of missing data on each variable ranged from 3% (Life Experiences Scale 1) to 22% (State Anxiety 2). Complete case analysis was used for each set of calculations. Spearman correlation coefficients were examined among continuous psychosocial measures and stress hormones. The association between psychosocial measures and other factors known to be associated with PTB[31] was then examined using *t*-tests for dichotomous variables, analysis of variance (ANOVA) for categorical variables, and correlations for continuous factors. This included demographic variables (age, income as a percent of the poverty line given reported household size, education, race, parity, and marital status), lifestyle variables (smoking, pre-pregnancy body mass index [BMI]), as well as pregnancy complications (preeclampsia, pregnancy-induced hypertension, anemia, history of PTB or miscarriage). Finally, we predicted biomarkers using linear regression based on these variables. Models included a quadratic term to examine possible non-linear relationships. Variables were modeled in the forms shown in Tables 2 and 3; psychosocial variables were modeled as continuous variables. Because cortisol and CRH increase with gestational age,[32,33] we also examined these models adjusted for gestational age.

We then examined whether associations between psychosocial measures and hormones changed when these other predictors of PTB were included in the models. Partial correlations were examined, and individual linear models were created, predicting biomarkers by incorporating these variables as well as psychosocial stress variables. We also examined these data using hierarchical linear models to control for the correlation within women. We examined whether the degree of correlation was affected by the time between questionnaire and the blood draw, by examining correlation within groups stratified by time between measurements. We also examined whether John Henryism, social support, and pregnancy-specific anxiety were effect modifiers of the psychosocial stress-stress hormone relationship by including a product term in the models.

Results

Most women in the PIN study were married and well-educated (Table 2). In this analysis, 20% were African-American, 14% smoked during pregnancy, and 13% had a household income below poverty level; 1,587 had at least one biological and one psychological stress measurement, while 716 had at least one cortisol measurement at 8–10 a.m. Median cortisol at first visit (median gestational week 17) was 0.40 μg/dl (standard deviation [SD] 0.24) and at the second visit (median gestational week 26) was 0.55 μg/dl (SD 0.26). Median CRH at first visit was 416 pg/mL (SD 268) and 511 pg/mL at the second visit (SD 368).

Life events, perceived stress, state anxiety, trait anxiety, and pregnancy-related anxiety were all positively correlated with one another, with correlation coefficients in the range of 0.2–0.6 (see online Supplementary Material at

Table 2. Characteristics of Women Participating in the Pregnancy, Infection, and Nutrition Study, 2000–2004

	At least one biological and one psychological marker of stress (n = *1587*)		*At least one cortisol measurement taken at 8:00–10:00 a.m.* (n = *716*)	
	N[a]	%	N	%
Age, years				
<20	132	8	53	7
20–24	298	19	136	19
25–29	505	32	236	33
30–35	460	29	215	30
>35	192	12	76	11
Race[a]				
White	1119	71	536	75
Black	324	20	128	18
Other	142	9	51	7
Parity				
0	731	46	347	48
1	549	35	248	35
2	211	13	82	11
3+	95	6	39	5
Education				
Less than high school	128	8	45	6
High school diploma	240	15	96	13
Some college	308	19	136	19
College degree	433	27	201	28
More than college	484	31	238	33
Marital status				
Single	345	22	145	20
Married	1176	74	545	76
Divorced/separated/widowed	63	4	25	4
Income				
<100	195	13	83	12
100–200	206	14	86	13
200–400	383	25	170	25
>400	712	48	345	50
Smoked during pregnancy				
Yes	207	14	80	12
No	1250	86	588	88
Working or fulltime student during pregnancy				
Yes	1284	82	600	85
No	278	18	106	15
Body mass index (Institute of Medicine categories)				
Underweight	215	14	102	14
Normal	793	51	352	50
Overweight	167	11	72	10
Obese	390	25	185	26

[a]Data do not add to column due to missing data.

www.liebertonline.com). Social support and John Henryism active coping were inversely correlated with the measures of perceived stress, life events, and anxiety. Cortisol and CRH were essentially uncorrelated with each other ($r < 0.06$).

Reported stress and biomarkers of stress were not closely correlated (Table 3), with correlation coefficients largely below $r = 0.1$. This was true for cross-sectional (visit 1 × visit 1), as well as lagged (visit 1 × visit 2) correlations. Higher levels of several measures of stress were associated with reduced cortisol or CRH. When biological measures were modeled as functions of psychosocial measures and gestational age, conclusions were similar to those from the correlation coefficients (data not shown). Correlations were not different among smokers and non-smokers.

Demographic and lifestyle variables were strongly predictive of psychosocial measures (see online Supplementary Material at www.liebertonline.com). Women who were younger, who were African-American, had less than a high school education, or smoked during pregnancy generally reported more stress, all types of anxiety, and less social support. Women with most medical risk factors later in pregnancy reported more stress and anxiety, except that pregnancy-induced hypertension (not pre-eclampsia) was associated with reporting fewer life events, lower state anxiety, and more social support.

Associations between demographic and lifestyle factors and biomarkers of stress were less clear (Table 4). Cortisol was highest in women who were young (difference between

Table 3. Spearman Correlations (r) among Psychosocial Measures and Biomarkers of Stress in North Carolina Pregnant Women, 2000–2004

	Log visit 1 cortisol[a]	*Log visit 2 cortisol*[b]	*Log visit 1 CRH*	*Log visit 2 CRH*
Negative life events, 1	−0.073	−0.041	−0.014	−0.046
Negative life events, 2	−0.127[c]	−0.031	−0.030	−0.053
Perceived stress 1	−0.040	−0.023	0.023	−0.047
Perceived stress 2	−0.070[c]	−0.074	−0.027	−0.063[c]
Anxiety 1	−0.042	0.018	−0.015	−0.037
Anxiety 2	0.006	0.009	−0.016	−0.083
Trait anxiety	−0.012	−0.024	0.000	−0.079
Total social support	0.085	0.093	0.006	0.023
Pregnancy-related anxiety 1	0.012	−0.025	0.015	−0.019
Pregnancy-related anxiety 2	−0.013	0.015	0.012	−0.042
Rini pregnancy-related anxiety	0.031	0.003	0.048	0.015
John Henryism	0.001	−0.007	−0.016	−0.023

[a]Visit 1, 14–18 weeks' gestation; 451 cortisol and 1486 CRH measures.
[b]Visit 2, 24–29 weeks' gestation; 353 cortisol and 1466 CRH measures.
[c]$p < 0.05$.
CRH, corticotropin-releasing hormone; SAQ, self-administered questionnaire.

youngest and oldest = 0.09 μg/dl at visit 1, 0.04 μg/dl at visit 2), nulliparous (nulliparous to parity ≥3, 0.15 μg/dl at visit 1), did not have hypertension (0.11 μg/dl at visit 1, 0.20 μg/dl at visit 2), or had lower BMIs (underweight to obese, 0.14 μg/dl at visit 1, 0.20 μg/dl at visit 2). CRH was highest in women who were more educated (difference between highest and lowest, 108 pg/mL at visit 2), who were not obese (obese to underweight, 84 pg/mL at visit 2), who were not African-American (white-African American, 58 pg/mL at visit 2), or who had pre-eclampsia (196 pg/mL at visit 2). When all covariates were included in single models, the strongest predictors of cortisol at both visits were lower parity and lower BMI; the strongest predictors of CRH were pre-eclampsia, anemia, and education (see online Supplementary Material at www.liebertonline.com).

Median time between telephone interview and hormone measurements was 12.5 days, with 50% occurring within 2 weeks of each other. Median time between self-administered questionnaire and hormone measurement was 0.5 days, with 50% within 1 week of each other. Correlations were not stronger when the measurements were taken closer together in time (see online Supplementary Material at www.liebertonline.com), nor did they increase when time of day and gestational age at time of sampling were incorporated.

John Henryism, social support, and pregnancy-specific anxiety were examined as effect modifiers of the stressor-stress hormone relationship, but models of these associations did not support this hypothesis.

Conclusions

We found little association between reported psychosocial measures and biological measures of stress, measured between the early second trimester and the early third trimester. While social characteristics and some medical factors were strongly predictive of psychosocial measures, controlling for these covariates did not affect the results materially. The prospective (visit 1/visit 2) associations were not stronger than the cross-sectional ones. Several other studies have shown a similar lack of association.[10,12,14,16,17] Even in studies that reported a positive association, there was often a single strong association amid several weak or nonexistent ones.[10] For example, Sandman looked at 10 correlations between cortisol and CRH; only between measurement at 15 or 19 weeks' and 31 weeks' gestation were the correlations large (greater than $r = 0.2$) or statistically significant.[6] Glynn et al. reported that cortisol at 18–20 weeks was associated with CRH at 30–32 weeks, but only among African-American and Hispanic women.[34] (They also found lower levels of CRH in African-American women, as did we.) Hobel et al. reported that stress at time 1 was positively associated with CRH at time 2 among women who delivered preterm, but among women who delivered full at term, stress and CRH were negatively associated.[7] Petraglia et al. reported little association between CRH, cortisol, and perceived stress and theorized that the increased CRH late in pregnancy masked any psychological response.[10,17]

We also found some differences between cortisol and CRH measures. While neither hormone was strongly associated with markers of stress, the strongest predictors of cortisol at both visits were lower parity and lower BMI, while the strongest predictors of CRH were pre-eclampsia, anemia, and education

The biomarkers may not have been measured in sufficient detail. Many factors affect cortisol secretion, such as time of awakening and sleep during the day. Some work suggests that reactivity or profile are more important than absolute levels of cortisol. CRH is secreted in a pulsatile fashion,[17] and cortisol secretion has a diurnal rhythm, peaking shortly after rising from bed and falling throughout the day.[35] Measures other than absolute level, such as morning rise or flattened cycles, have been more strongly associated with health outcomes in some instances.[36] Obel et al. found evening cortisols to be more strongly associated with stress markers than morning cortisols.[13] Measurements later in the day might have reduced baseline variability and thus allow for better differentiation between groups. CRH may need to be examined in the context of its binding protein and the relative contributions of placental and hypothalamic CRH.[9] CRH trajectory, which needs more than two measurements to plot,

Table 4. Biomarkers of Stress by Demographic and Medical Factors in North Carolina Pregnant Women, 2000–2004

	Cortisol, 14–18 weeks' gestation (n = *460*)			*Cortisol, 24–29 weeks' gestation* (n = *353*)			*Corticotropin-releasing hormone, 14–18 weeks' gestation* (n = *1486*)			*Corticotropin-releasing hormone, 24–29 weeks' gestation* (n = *1466*)		
	Mean[a]	*SD*	p	*Mean*	*SD*	p	*Mean*[b]	*SD*	p	*Mean*	*SD*	p
Age, years			0.13			0.33			0.51			0.51
<20	0.52	0.29		0.51	0.20		484	257		616	385	
20–24	0.46	0.20		0.56	0.23		463	261		586	339	
25–29	0.42	0.22		0.56	0.26		480	274		613	364	
30–35	0.42	0.27		0.57	0.29		488	277		633	380	
>35	0.41	0.19		0.47	0.20		451	252		635	383	
Race			0.21			0.30			0.48			0.06
White	0.44	0.24		0.56	0.27		475	270		629	371	
Black	0.40	0.23		0.50	0.23		469	266		571	352	
Other	0.48	0.20		0.53	0.21		503	263		614	374	
Parity			0.00			0.12			0.73			0.51
0	0.48	0.25		0.58	0.27		469	261		628	386	
1	0.40	0.22		0.52	0.24		486	281		608	354	
2	0.41	0.22		0.53	0.21		478	257		620	354	
3+	0.33	0.12		0.48	0.32		472	275		570	344	
Education			0.11			0.34			0.29			0.01
Less than high school	0.47	0.32		0.51	0.24		424	239		543	375	
High school	0.37	0.23		0.49	0.22		483	262		567	319	
Some college	0.41	0.30		0.53	0.26		481	262		622	385	
College degree	0.46	0.21		0.58	0.31		475	273		619	360	
More than college	0.45	0.20		0.56	0.22		484	278		651	381	
Marital status			0.68			0.79			0.40			0.04
Single	0.43	0.25		0.53	0.22		476	272		573	357	
Married	0.44	0.24		0.55	0.26		473	267		626	371	
Divorced/separated/ widowed	0.39	0.16		0.58	0.28		523	280		674	373	
Income			0.35			0.86			0.50			0.46
<100	0.43	0.25		0.54	0.20		451	235		601	360	
100–200	0.39	0.22		0.56	0.29		491	280		613	400	
200–400	0.42	0.21		0.53	0.25		473	269		599	367	
>400	0.45	0.25		0.56	0.27		481	279		634	368	
Smoked during pregnancy			<0.01			0.79			0.72			0.58
Yes	0.36	0.17		0.56	0.26		466	261		598	371	
No	0.45	0.25		0.55	0.26		478	272		624	373	
Working or fulltime student during pregnancy			0.44			0.05			0.76			0.42
Yes	0.43	0.24		0.54	0.24		477	273		614	365	
No	0.46	0.22		0.62	0.35		471	252		634	386	
Body mass index (Institute of Medicine categories)			<0.01			<0.01			0.33			
Underweight	0.51	0.24		0.65	0.23		490	275		636	356	<0.01
Normal	0.46	0.21		0.57	0.27		478	273		638	389	
Overweight	0.40	0.21		0.52	0.20		495	266		628	356	
Obese	0.37	0.27		0.45	0.23		455	256		552	314	
Ethnicity			0.43			0.42			0.90			0.39
Hispanic	0.47	0.22		0.50	0.27		480	241		657	498	
Non-Hispanic	0.43	0.24		0.55	0.26		476	271		616	362	
Pre-eclampsia in this pregnancy			0.36			0.03			0.35			<0.01
Yes	0.39	0.17		0.42	0.18		503	264		802	552	
No	0.44	0.24		0.55	0.26		474	267		606	352	
Pregnancy-induced hypertension in this pregnancy			0.16			0.10			0.58			0.93
Yes	0.38	0.19		0.46	0.12		461	208		613	340	
No	0.44	0.24		0.55	0.26		477	271		617	370	
Chronic hypertension			<0.01			<0.01			0.41			0.72
Yes	0.33	0.22		0.36	0.15		456	271		604	357	
No	0.44	0.24		0.56	0.26		477	267		617	369	

(*Continued*)

Table 4. (Continued)

	Cortisol, 14–18 weeks' gestation (n = *460*)			*Cortisol, 24–29 weeks' gestation* (n = *353*)			*Corticotropin-releasing hormone, 14–18 weeks' gestation* (n = *1486*)			*Corticotropin-releasing hormone, 24–29 weeks' gestation* (n = *1466*)		
	Mean[a]	*SD*	p	*Mean*	*SD*	p	*Mean*[b]	*SD*	p	*Mean*	*SD*	p
Anemia, trimester 2			0.52			0.68			0.12			0.08
Yes	0.41	0.19		0.57	0.22		515	298		678	406	
No	0.44	0.24		0.54	0.26		473	264		612	364	
Previous miscarriage			0.35			0.29			0.21			0.32
0	0.44	0.23		0.55	0.26		469	261		610	354	
1	0.45	0.29		0.56	0.24		485	286		625	384	
2+	0.39	0.16		0.48	0.26		511	289		662	448	
Previous preterm birth			0.04			0.27			0.93			0.08
0	0.45	0.24		0.56	0.25		475	265		626	378	
1	0.36	0.23		0.48	0.26		479	301		581	312	
2+	0.37	0.15		0.56	0.35		488	248		533	288	
Exercise before pregnancy			0.05			0.39			0.12			0.32
Yes	0.46	0.25		0.57	0.28		446	249		647	379	
No	0.41	0.24		0.54	0.26		472	264		623	373	
Exercise in trimester 1			0.34			0.97			0.34			0.92
Yes	0.46	0.26		0.56	0.26		446	240		640	356	
No	0.43	0.23		0.56	0.28		462	264		637	392	

[a]in μg/dl; limited to samples provided at 8–10 a.m.
[b]in pg/ml.
SD, standard deviation.

may differentiate more accurately among different types of PTB.[37] A different timing of measurement during gestation may be more relevant. For instance, one study found that stress early in pregnancy was most important with later responses to stress being muted,[38] while other studies have focused on the third trimester[12,39] and still others suggest the pattern of stressors is most important.[40] The rise in both cortisol and CRH during pregnancy may swamp any smaller increase due to stress.

However, data were collected by an experienced and organized team using currently accepted instruments and procedures. Specimens were handled according to guidelines and assays conducted by experienced laboratories. Multiple measures on multiple days could provide a more rigorous measure of parameters such as reactivity and cortisol profile, but are not practical for most large-scale, population-based studies. Research indicates that CRH[41] and cortisol[42] are both fairly stable and that assays are accurate even after delayed processing. Important research in perinatal epidemiology has used single cortisol measurements,[12,18] prompting our examination.

Standardized stress measures reflect perceptions that can change over time and perhaps particularly during a pregnancy. More detailed interviews provide more information on stressors and psychological state, and not all questionnaires are understood by every population. The Sarason life events questionnaire does not include many possibly stressful life events. However, several studies indicate that chronic stress is more predictive of poor outcome than short-term stress, even quite severe stresses.[43] Measures that concentrate on the woman's immediate circumstances may thus not be predictive. These instruments to measure the presence of psychosocial stress may be inadequate to address the construct of interest. Chronic stressors such as racism, disadvantage, or poverty may actually be better reflected in demographic markers than in ostensibly more direct instruments. For example, life events questionnaires alone may underestimate the differences in experience between whites and African-Americans, as well as women with different social and economic resources.[44]

Researchers examining the topic of stress in pregnancy need to be aware of the limitations inherent in the measurements of these phenomena and hypothesized pathways. Empirical support for the model in which psychosocial stressors stimulate production of stress hormones, cortisol, and CRH, which in turn are the direct cause of preterm birth, is limited. While cell culture studies have elucidated mechanisms of hormonal action, the transfer to whole organisms is less clear. If CRH only reflects fetal stress or physiological stress, such evidence does not bear on the relationship between mental states and pregnancy outcome, nor does it provide an avenue for understanding health disparities, though it may be useful as a predictor or marker of high risk. On the other hand, if stress does raise cortisol and CRH and this results in PTB, then our techniques for measuring reported stress are falling short. Future studies need to examine refined measures of biomarkers, novel biomarkers, and demonstrate that the proposed causal pathway is operative in order to make progress in identifying promising interventions.

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